Development and validation of simultaneous estimation of diclofenac potassium, paracetamol and serratiopeptidase by first order derivative UV spectroscopy method in pharmaceutical formulation

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ABSTRACT

The objective of present study was to develop simple, accurate, precise, cost effective and reliable analytical method for estimation of Diclofenac potassium (DCL), Serratiopeptidase (SER) and Paracetamol (PCM) in combined tablet dosage form. First order derivative simultaneous estimation was carried out by using UV visible double beam spectrophotometer. Developed method uses a solvent methanol: distiied water (40:60) for wavelength determination. Sample was scanned at 200-400 nm and working wavelength (252 nm, 276 nm and 330 nm) was detected. Validation of simultaneous spectrophotometric methods was performed with different parameters. In first order derivative method, at 252 nm, 276 nm SER, at 330 nm DCL and at 252 nm, 276 nm PCM showed significant absorbance. Linearity of SER, DCL and PCM was 2 µg/ml to 15 µg/ml, 2 µg/ml to 30 µg/ml and 2 µg/ml to 80 µg/ml respectively. The method precisions were exemplified by relative standard deviations of 1.82% and 1.77% for SER, 1.42% and 1.42% for DCL and 1.60% and 1.82% for PCM in intra and inter day precision respectively. The %R.S.D. is less than 2% as required by USP and ICH guidelines. Present study revealed that developed and validated first order derivative UV spectroscopy method for simultaneous estimation of SER, DCL and PCM was found to be least time consuming, accurate, precise and linear.
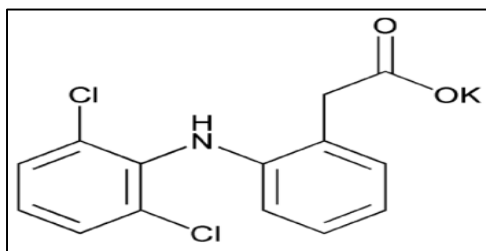
Keywords: UV spectrophotometer, Diclofenac potassium, Paracetamol, Serratiopeptidase, Simultaneous spectrophotometric method, Validation
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INTRODUCTION

Diclofenac potassium (DCL) is (Benzene acetic acid, 2-[(2, 6 dichlorophenyl) amino]-monopotassium salt) [1] non-steroidal anti-inflammatory drug which inhibits leukocyte migration and the enzyme cyclooxygenase (COX-1 and COX-2) [2-5]. Paracetamol (PCM) (4-hydroxyacetanilide) [6] non-steroidal anti-inflammatory drug which inhibits isoforms of cyclooxygenase, COX-1, COX2 and COX-3 enzymes involved in prostaglandin (PG) synthesis [7-10] and Serratiopeptidase (SER) is Proteolytic enzyme [11]which produce hydrolysis of histamine, bradykinin and serotonin, thus produce anti-inflammatory effect [12].

A tablet dosage from containing all the three, (DCL 15mg, SER 15mg and PCM 325), is commercially available and used in pain reliever and reducer for inflammation.

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Structure of Diclofenac potassium   Structure of Paracetamol
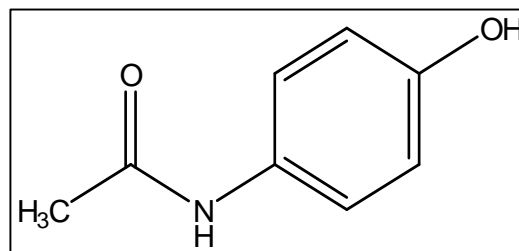
DCL official in USP/NF. SER and PCM are officially available in IP. Literature reviews revealed that UV Spectroscopy method have been reported for the determination of Diclofenac potassium, Serratiopeptidase and Paracetamol individually in pharmaceutical dosage form. Literature is also available concerning UV Spectroscopy method of Diclofenac potassium and Paracetamol with drug combination [13-33]. As per our detailed survey on date, there are only one to two methods available for Serratiopeptidase [34-36].But; there is no any UV Spectroscopy method available for Diclofenac potassium, Serratiopeptidase and Paracetamol in pharmaceutical dosage form. So, present study was aimed at to develop simple, accurate, precise, cost effective and reliable analytical method for estimation of Diclofenac potassium, Paracetamol and Serratiopeptidase in combined tablet dosage form.

For the estimation of multicomponent sample in UV spectrophotometer, different spectrophotometric simultaneous estimation methods are used as follow:[37]

Absorbance correction method, assay as a single-component sample, assay using absorbance corrected for interference, simultaneous equation method, absorbance ratio method,geometric correction method, orthogonal polynomial method, difference spectrophotometry, derivative spectrophotometry and least square approximation [37].

EXPERIMENTAL SECTION

Chemical and Reagent
Analytically pure sample with purities greater than 99% were obtained as gift samples of Diclofenac potassium (Tripada Pharmaceuticals Private Limited, Ahmedabad), Serratiopeptidase (J C Biotech Private Limited, Hydrabad) and Paracetamol (Farmason Pharmaceutical Gujarat Private Limited, Vadodara). Methanol was procured from Molychem, Mumbai. Distilled water was prepared inhouse. Methanol:distilled water (40:60) was used as a solvent in which all three drug are very much stable.

Instrument
UV Spectroscopy was performed with UV software by using UV Visible Spectrophotometer (Labtronic LT2900-Double beam). An electronic analytical weighing balance (Dhona) and sonicator were used in this study.

UV Spectroscopy
Preparation of standard stock solution
10 mg of Diclofenac potassium, 20 mg of Paracetamol and 10 mg of Serratiopeptidase were weighed separately and transferred in three different 100ml volumetric flasks. All the drugs were dissolved in 50 ml of solvent by vigorous shaking and then volume was made upto the mark with solvent to obtain final concentration of 100 µg/ml of Diclofenac potassium, 200 µg/ml of Paracetamol and 100 µg/ml of Serratiopeptidase.

Here, solution of Serratiopeptidase was prepared and maintained in a dark room condition throughout experimental work.

Selection of Analytical wavelength
Using appropriate dilutions of the standard stock solution, the solutions were scanned separately in the wavelength region of 400–200nm. The absorbance spectra, thus obtained were derivatized to remove the interference of absorbing species (Fig. 1, 2, 3). By using overlay spectra of three drugs, working wavelengths selected were 252

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nm, 276 nm and 330 nm (Fig. 4). From the examination of the overlain first derivative spectra, it was observed that Serratiopeptidase and Paracetamol showed absorbance at 252 nm and 276 nm, whereas absorbance of Diclofenac potassium was found at 330 nm. Absorbance of Serratiopeptidase and Paracetamol was zero at 330 nm. So, 252 nm and 276 nm were selected as working wavelengths for Serratiopeptidase and Paracetamol and for Diclofenac potassium working wavelength selected was 330 nm for first derivative spectroscopy (Fig. 1, 2, 3).

Selection of analytical concentration range

For each drug appropriate aliquots were pipetted out from the standard stock solutions into series of 10 ml volumetric flask. The volume was made upto the mark with solvent to get a set of solutions having the concentration of 2, 5, 10, 15, 20 µg/ml for Serratiopeptidase, 2, 5, 10, 20, 30 µg/ml for Diclofenac potassium and 2, 5, 10, 20, 30, 40, 50, 60 µg/ml for Paracetamol. The absorbance of derivatized spectrum of each of these solutions were measured at the selected wavelengths (for Serratiopeptidase and Paracetamol at 252 nm and 276 nm, for Diclofenac potassium at 330 nm) and plotted against concentration. The concentration range over which the drugs obeyed Beer's law was chosen. The range was found to be 2.0 - 20.0 µg/ml for Serratiopeptidase, 2.0 - 30 µg/ml for Diclofenac potassium and 2.0 - 60.0 µg/ml for Paracetamol. (Table 1, 2 and 3) (Fig. 5, 6, 7, 8, 9).

Procedure for analysis of powder mixture

Mixed solution of pure drugs was prepared by taking suitable volume of standard drug solution. Here, 1.0 ml standard solution of Serratiopeptidase (100 µg/ml), 1.731 ml standard solution of Diclofenac potassium (100 µg/ml) and 5.0 ml standard solution of Paracetamol (200 µg/ml) were transferred into 10 ml volumetric flask to make final concentration of 10 µg/ml for Serratiopeptidase, 17.31 µg/ml for Diclofenac potassium and 100 µg/ml for Paracetamol. Absorbance of derivatized spectrum of this prepared mixed solution was measured at 252 nm, 276 nm and 330 nm (Fig 10).

The values were substituted in equation (28) and (29) to get a concentration of Serratiopeptidase and Paracetamol respectively (Fig. 33). Amount of Diclofenac potassium was determined by using regressin equation (y = -0.0002x – 0.0002) of Diclofenac potassium plotted at 330 nm.

Results of the analysis of powder mixture are reported in (Table 4 and 5).

$$C_{PCM} = \frac{A_1 \times (-0.18) - A_2 \times (-0.18)}{(-1.28 \times -0.18) - (-0.92 \times -0.18)}$$

$$C_{SER} = \frac{A_2 \times (-1.28) - A_1 \times (-0.92)}{(-1.28 \times -0.18) - (-0.92 \times -0.18)}$$

Where
1. (-1.28) and (-0.92) are absorptivities of PCM at 276 λ_1 and 252 λ_2 respectively.
2. (-0.18) and (-0.18) are absorptivities of SER at 276 λ_1 and 252 λ_2 respectively.
3. A_1 (-0.0658) and A_2 (-0.0478) are absorbances of mixtures at 276 λ_1 and 252 λ_2 respectively.
4. C_{PCM} and C_{SER} are concentrations in gm / liter.

Procedure for analysis of tablet formulation

Twenty tablets of Serratiopeptidase, Diclofenac potassium and Paracetamol in combination were weighed and their average weight was determined and the tablets were crushed to powder sample. Average weight of tablet was found to be 466.3 mg. From the triturate, 7.173 mg tablet powder (equivalent to 0.23 mg of Serratiopeptidase, 0.7692 mg of Diclofenac potassium and 5 mg of Paracetamol) was weighed and transferred into 50 ml volumetric flask and dissolved in solvent and the content was kept in ultrasonicator for 20 min. To this volumetric flask, 2.692 ml standard stock solution of Serratiopeptidase (100 µg/ml) was added. Finally the volume was made upto the mark with solvent. The solution was filtered through Whatman filter paper No.41. Volume was made upto the mark with solvent to make final concentration of 10 µg/ml for Serratiopeptidase, 17.31 µg/ml for Diclofenac potassium and 100 µg/ml for Paracetamol. The mixed sample solutions were scanned to obtain spectra. The absorbance of derivatized spectrum of the solution at 252 nm, 276 nm and 330 nm were measured (Fig 11). The values were substituted in equation (28 and 29) to get a concentration of Serratiopeptidase and Paracetamol respectively. Amount of Diclofenac potassium was determined by using regress in equation (y = -0.0002x – 0.0002) of Diclofenac potassium plotted at 330 nm.

The results of the analysis of tablet formulation are reported in Table 6 and 7.

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Procedure for recovery studies

Recovery studies were carried out by applying the method to drug sample present in tablet dosage form to which known amount of Diclofenac potassium, Paracetamol and Serratiopeptidase corresponding to 80, 100, 120% of label claim was added (standard addition method). In 80% recovery study, amount of standard added is 6 mg of Serratiopeptidase, 20 mg of Diclofenac potassium and 130 mg of Paracetamol in a half of the average weight of tablet triturate (i.e., 233.15 mg). In 100% recovery study, amount of standard added is 7.5 mg of Serratiopeptidase, 25 mg of Diclofenac potassium and 162.5 mg of Paracetamol in a half of the average weight of tablet triturate (i.e., 233.15 mg). In 120% recovery study, amount of standard added is 9 mg of Serratiopeptidase, 30 mg of Diclofenac potassium and 195 mg of Paracetamol in a half of the average weight of tablet triturate (i.e., 233.15 mg). After the addition of the standards the tablet triturates were mixed properly. From this tablet and standard mixture, mixed powder (equivalent to 0.23 mg of Serratiopeptidase, 0.7692 mg of Diclofenac potassium and 5 mg of Paracetamol) was weighed and transferred into 50 ml volumetric flask and dissolved in solvent and the content was kept in ultrasonicator for 20 min. Finally the volume was made uptothe mark with solvent. The solution was filtered through Whatman filter paper No.41. From this solution suitable amount was transferred into another 50 ml volumetric flask and required volume of standard stock solution of Serratiopeptidase (100 µg/ml) was added in such a way that final solution to be analysed contains 10 µg/ml Serratiopeptidase, 17.31 µg/ml Diclofenac potassium and 100 µg/ml Paracetamol.

The mixed sample solutions were analysed to obtain spectra and absorbance value of derivatized spectrum at 252 nm, 276 nm and 330 nm (selected wavelength of Diclofenac potassium, Paracetamol and Serratiopeptidase) were noted (Fig 11).

The concentrations were determined as per the procedure given for the tablet formulation. At each level, three determinations were performed and results obtained were compared with expected results. The results for recovery studies and statistical evaluation data are shown in Table 8 and 9.

Procedure for Precision

Precision of the method was determined with the tablet sample. Twenty tablets of Serratiopeptidase, Diclofenac potassium and Paracetamol in combination were weighed and their average weight was determined and the tablets were crushed to fine powder. Average weight of tablet was found to be 466.3 mg. From the triturate, 7.173 mg tablet powder (equivalent to 0.23 mg of Serratiopeptidase, 0.7692 mg of Diclofenac potassium and 5 mg of Paracetamol) was weighed and transferred into 50 ml volumetric flask and dissolved in solvent and the content was kept in ultrasonicator for 20 min. To this volumetric flask, 2.692 ml standard stock solution of Serratiopeptidase (100 µg/ml) was added. Finally the volume was made upto the mark with solvent. The solution was filtered through Whatman filter paper No.41. Volume was made uptothe mark with solvent to make final concentration of 10 µg/ml for Serratiopeptidase, 17.31 µg/ml for Diclofenac potassium and 100 µg/ml for Paracetamol.

The mixed sample solutions were analysed to obtain spectra and absorbance value of derivatized spectrum at 252 nm, 276 nm and 330 nm were noted. The concentration of Serratiopeptidase, Diclofenac potassium and Paracetamol were calculated from the equation. In intraday precision, sample having concentration of 10 µg/ml of Serratiopeptidase, 17.31 µg/ml of Diclofenac potassium and 100 µg/ml of Paracetamol was scanned six times at different time interval in the same day. Interday precision was obtained by the assay of six sample sets on different days as per the same procedure. The results are shown in (Table 10, 11, 12 and 13).

<div align="center">

RESULTS

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Analytical working wavelength

From the examination of the overlay first derivative spectra of Serratiopeptidase and Paracetamol gave absorbance at 252 nm and 276 nm. Absorbance of Serratiopeptidase and Paracetamol was zero at 330 nm. The absorbance of Diclofenac potassium was found at 330 nm and absorbance of Diclofenac potassium shown zero at 252 nm and 276 nm. So, working wavelengths for Serratiopeptidase and Paracetamol were selected 252 nm and 276 nm and for Diclofenac potassium working wavelength were selected 330 nm for first derivative spectroscopy (Fig. 1, 2, 3 & 4).

Result for analytical concentration range

The range was found to be 2, 5, 10, 15 µg/ml for Serratiopeptidase, 2, 5, 10, 20, 30 µg/ml for Diclofenac potassium and 2, 5, 10, 20, 30, 40, 50, 60 µg/ml for Paracetamol. The working curve equation was found to be for

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Serratiopeptidase y=-0.0002x-8E-05 with correlation coefficient (r^2) value of 0.9944 and y=-0.0002x-1E-04 with correlation coefficient (r^2) value of 0.9958 on 252 nm and 276 nm respectively. For Diclofenac potassium y=-0.0002x-0.0002 with correlation coefficient (r^2) value of 0.9959 on 330 nm. For Paracetamol y=-0.0009x-0.0022 with correlation coefficient (r^2) value of 0.996 and y=-0.0012x-0.0038 with correlation coefficient (r^2) value of 0.9925 on 252 nm and 276 nm respectively (Table 1, 2 & 3 and Fig. 7, 8, 9, 10 & 11).
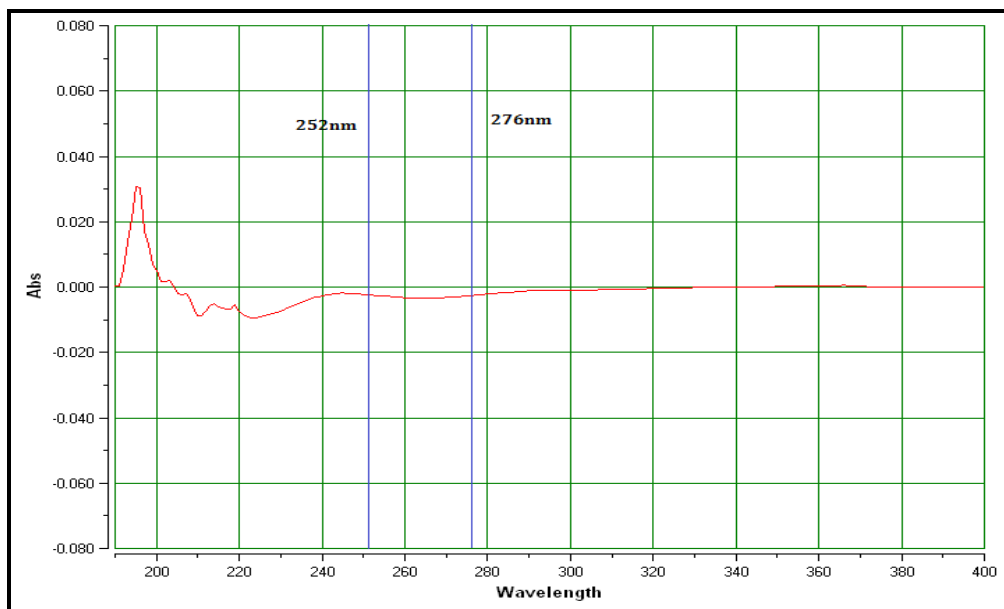

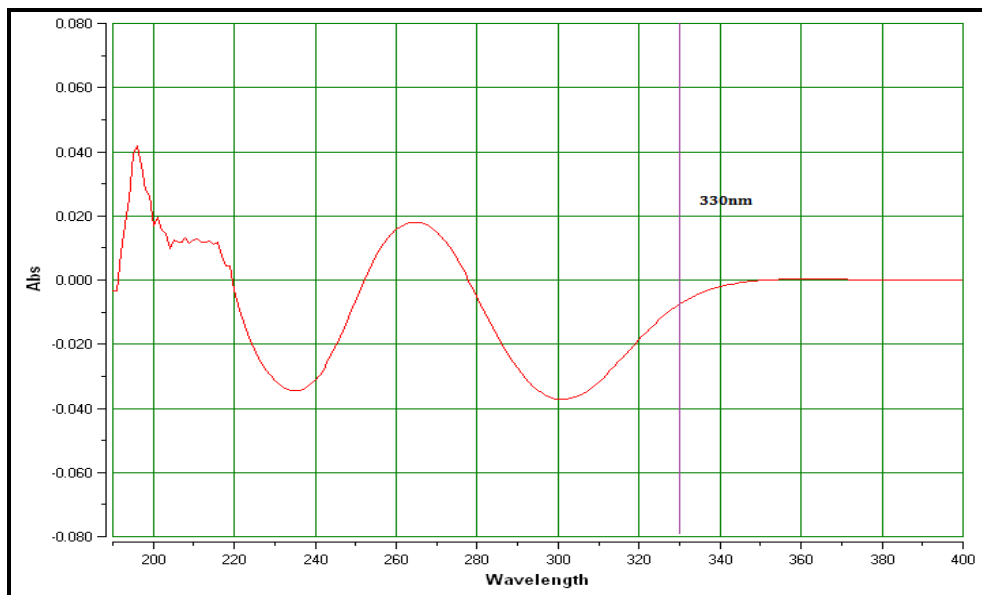**Figure 1. First Derivative Spectrum of Serratiopeptidase**

Figure 2. First Derivative Spectrum of Diclofenac potassium

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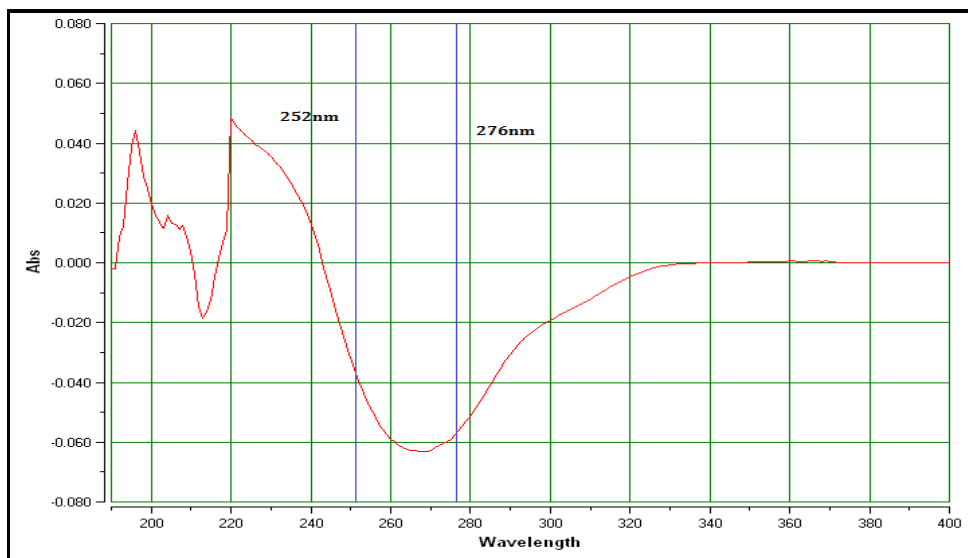
Figure 3. First Derivative Spectrum of Paracetamol
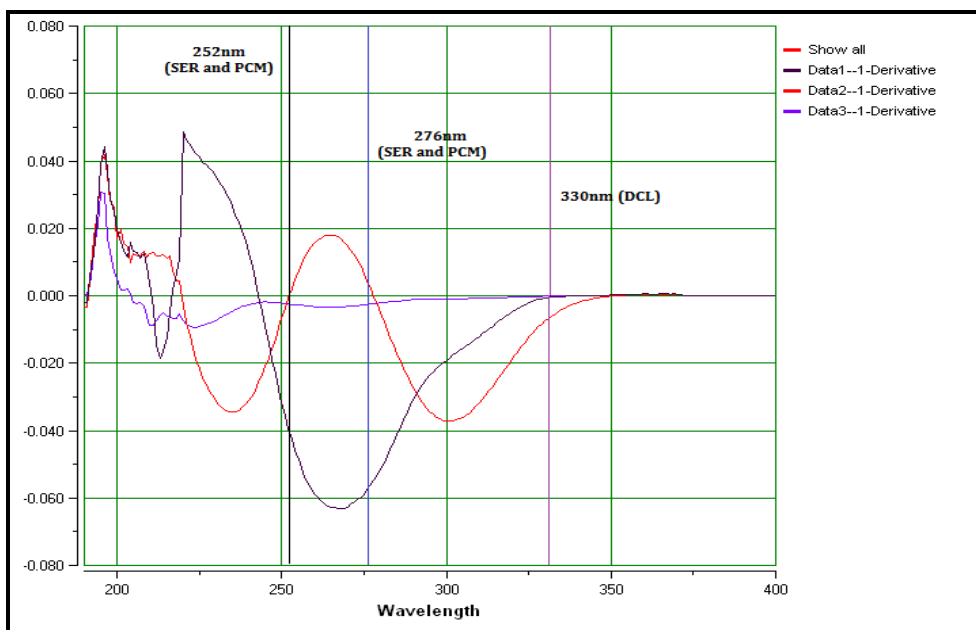
Figure 4. Overlay Spectrum of Serratiopeptidase, Diclofenac potassium and Paracetamol of First Derivative

Table 1. Calibration Curve for Serratiopeptidase of First Derivative

Sr. No.	Concentration (µg/ml)	Absorbance at 252 nm	Absorbance at 276 nm
1	2	-0.0004	-0.0005
2	5	-0.001	-0.001
3	10	-0.0018	-0.0018
4	15	-0.0025	-0.0026

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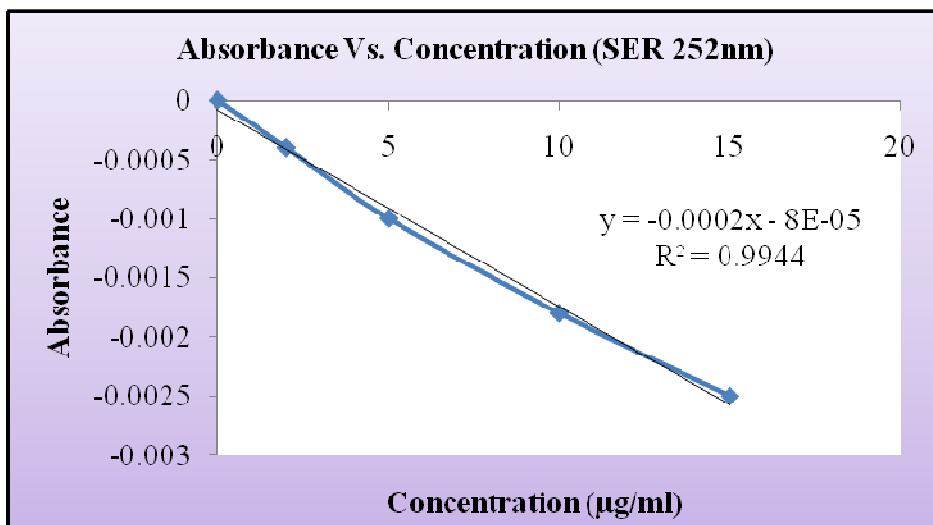
Figure 5. First Derivative Calibration Curve for Serratiopeptidase at 252 nm

Figure 6. First Derivative Calibration Curve for Serratiopeptidase at 276 nm

Table 2. Calibration Curve for Diclofenac potassium of First Derivative

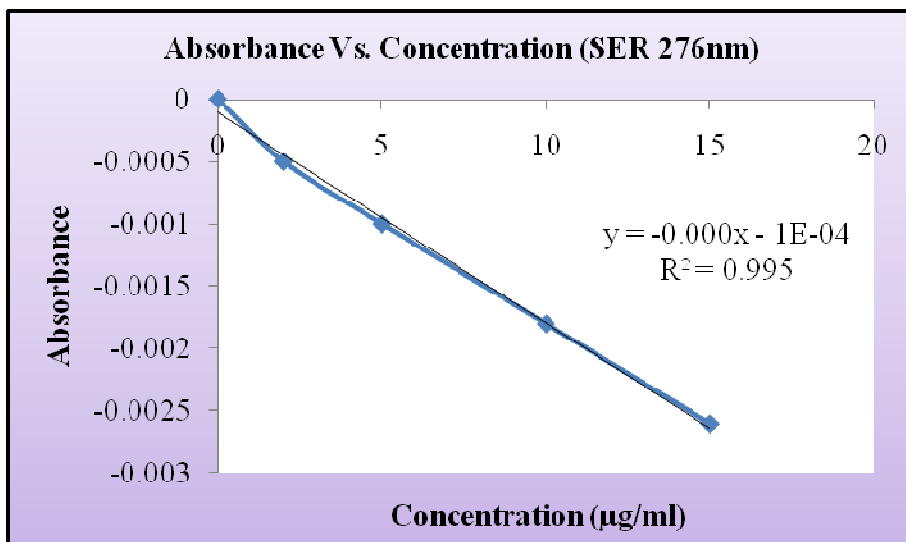| Sr. No.	Concentration(μg/ml)	Absorbance at 330 nm
1	2	-0.0007
2	5	-0.0012
3	10	-0.0023
4	20	-0.0042
5	30	-0.0059

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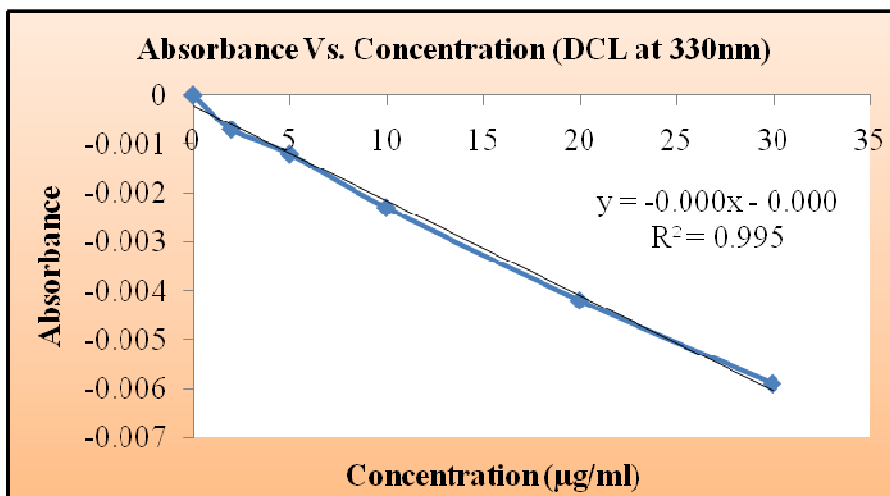
Figure 7. First Derivative Calibration Curve for Diclofenac potassium at 330 nm

Table 3. Calibration Curve for Paracetamol of First Derivative Spectroscopy

Sr. No.	Concentration (µg/ml)	Absorbance at 252 nm	Absorbance at 276 nm
1	2	-0.0038	-0.0055
2	5	-00082	-0.0117
3	10	-0.011	-0.016
4	20	-0.022	-0.029
5	30	-0.030	-0.043
6	40	-0.038	-0.054
7	50	-0.046	-0.064
8	60	-0.057	-0.073
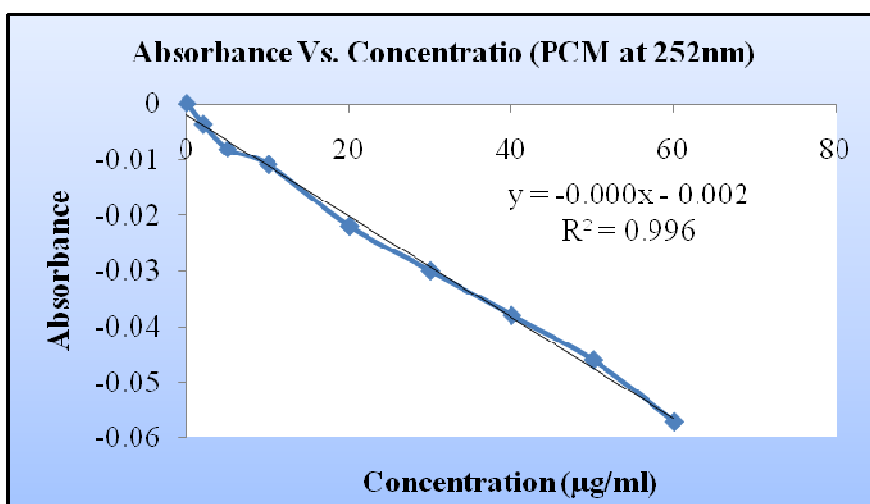
Figure 8. First Derivative Calibration Curve for Paracetamol at 252 nm

Analysis of powder mixture:

First order derivative spectrum of Powder Mixture was scanned with three different wavelengths (Fig. 10). Results of the analysis of powder mixture were reported Table 4 and 5.

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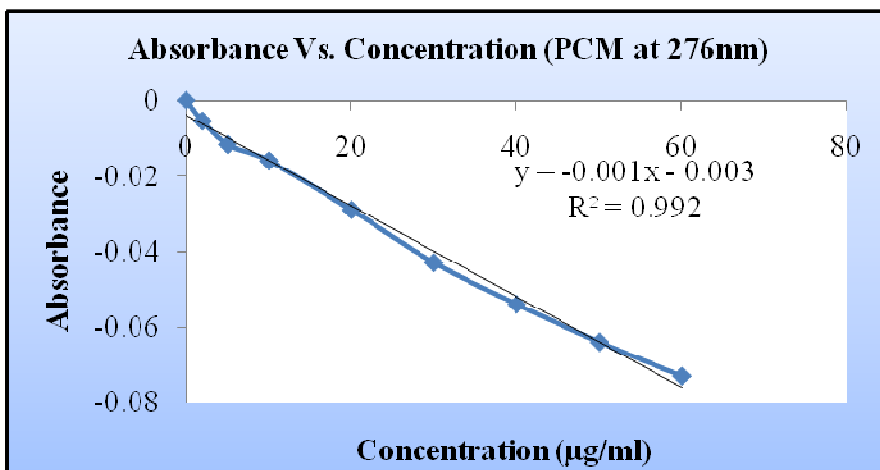
Figure 9. First Derivative Calibration Curve for Paracetamol at 276 nm
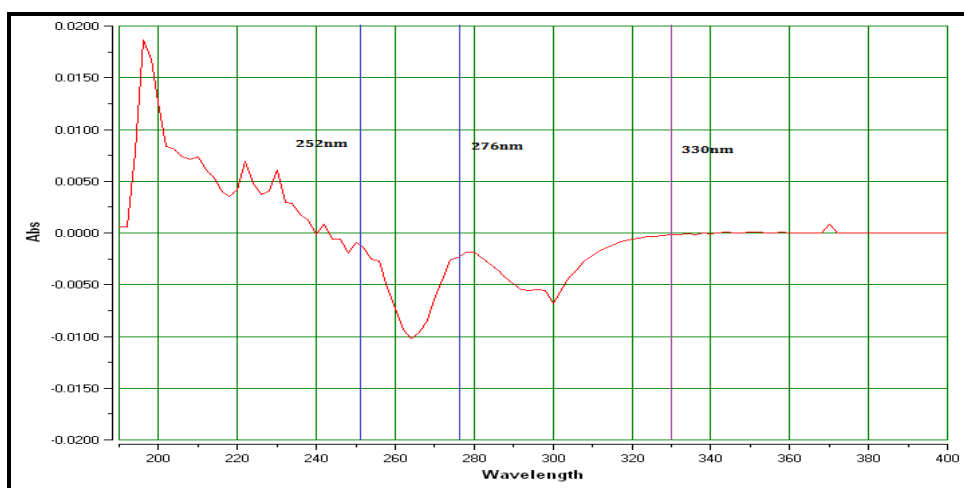
Figure 10.First Order Derivative Spectrum of Powder Mixture

Table 4. Analysis for Powder Mixture

Sr. No.	Amount present (µg/ml)			Amount found (µg/ml)			Amount found (%)		
	SER	**DCL**	**PCM**	**SER**	**DCL**	**PCM**	**SER**	**DCL**	**PCM**
1	10	17.308	100	9.86	17	100	98.6	98.22	100
2	10	17.308	100	9.93	17	99	99.3	98.22	99
3	10	17.308	100	9.97	17.5	104.33	99.7	101.10	104.33
4	10	17.308	100	10.07	17.5	101.19	100.7	101.10	101.19
5	10	17.308	100	10.10	17.5	99.72	101.0	101.10	99.72
6	10	17.308	100	10.30	17.5	100	103.0	101.10	100

Table 5. Statistical Validation for Powder Mixture

Component	Mean*	Standard Deviation*	Co-efficient of Variation*	Standard Error*
SER	10.03	0.155	1.545	0.063
DCL	96.775	1.582	1.634	0.645
PCM	100.70	1.910	1.896	0.779

* n=6

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Analysis of tablet formulation

First order derivative spectrum of tablet formulation was scanned with three different wavelengths (Fig. 11). Results of the analysis of tablet formulation were reported (Table 6 & 7).
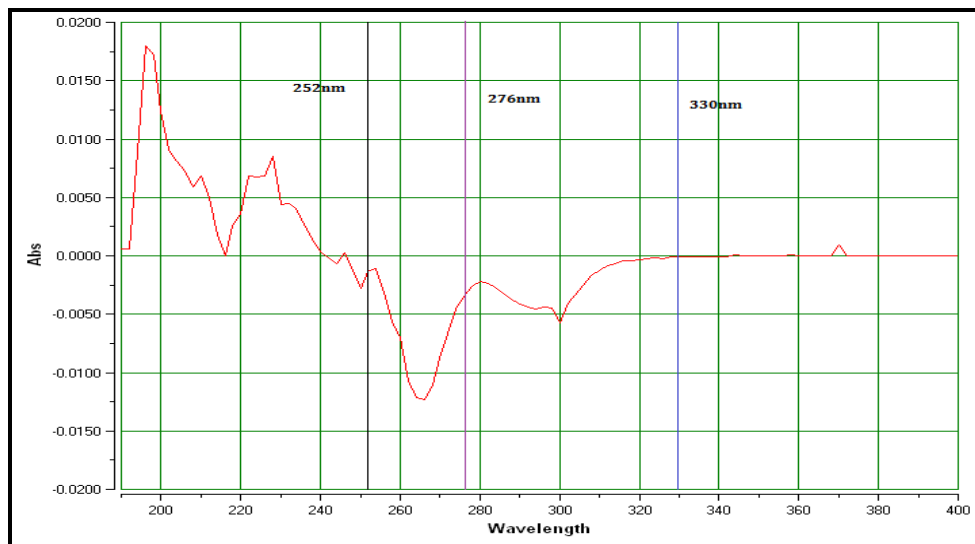

Figure 11. First Order Derivative Spectrum of tablet Mixture

Table 6. Analysis of Tablet Formulation

Sr. No.	Amount present in μg/ml			Amount found in μg/ml			Amount found in %		
	SER	DCL	PCM	SER	DCL	PCM	SER	DCL	PCM
1	10	17.308	100	10.10	17	100	101	98.22	100
2	10	17.308	100	9.86	17	99	98.6	98.22	99
3	10	17.308	100	9.93	17.5	99.72	99.3	101.10	99.7
4	10	17.308	100	10.07	17.5	100	100.7	101.10	100
5	10	17.308	100	10.30	17.5	104.33	103.0	101.10	104.33
6	10	17.308	100	9.97	17.5	101.99	99.7	101.10	101.99

Table 7. Statistical Validation for the Tablet Formulation

Component	Mean*	Standard Deviation*	Co-efficient of Variation*	Standard Error*
SER	100.38	1.558	1.552	0.636
DCL	101.14	1.487	1.470	0.607
PCM	100.83	1.979	1.932	0.808

* n=6

Recovery studies

The results for recovery studies and statistical evaluation data were reported (Table 8 & 9).

Table 8.Recovery Studies

Level of % Recovery	Amount Present in tablet (mg/ml)			Amount Present in prepared solution(μg/ml)			Standard Amount Added in solution (μg/ml)			Total Amount Recovered (μg/ml)			% Recovery		
	SER	DCL	PCM	SER	DCL	PCM	SER	DCL	PCM	SER	DCL	PCM	SER	DCL	PCM
80	15	50	325	4.616	17.308	100	3.692	13.84	80	8.8	31.0	178	103.51	99.52	98.88
80	15	50	325	4.616	17.308	100	3.692	13.84	80	8.3	31.5	179	99.90	101.13	99.44
80	15	50	325	4.616	17.308	100	3.692	13.84	80	8.5	32.0	183	102.31	102.76	101.66
100	15	50	325	4.616	17.308	100	4.616	17.308	100	9.0	34.0	199	97.48	98.22	99.50
100	15	50	325	4.616	17.308	100	4.616	17.308	100	9.3	34.5	205	100.71	99.66	102.50
100	15	50	325	4.616	17.308	100	4.616	17.308	100	9.2	35.5	206	99.65	101.10	103.00
120	15	50	325	4.616	17.308	100	5.539	20.76	120	10.17	38.0	221	100.14	99.87	100.45
120	15	50	325	4.616	17.308	100	5.539	20.76	120	10.26	38.5	226	101.63	101.18	102.72
120	15	50	325	4.616	17.308	100	5.539	20.76	120	10.55	39.0	228	103.88	102.50	103.63

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Table 9. Statistical Validation for Recovery Studies

Level of % Recovery	% Mean Recovery*			Standard Deviation*			Co-efficient of Variation*			Standard Error*		
	SER	DCL	PCM	SER	DCL	PCM	SER	DCL	PCM	SER	DCL	PCM
80	101.90	101.13	99.99	1.838	1.620	1.476	1.803	1.601	1.476	0.750	0.661	0.602
100	99.28	99.66	101.66	1.646	1.440	1.892	1.657	1.444	1.861	0.672	0.587	0.772
120	101.88	101.18	102.26	1.953	1.315	1.637	1.916	1.299	1.600	0.797	0.536	0.668

n=6

Precision:
The results of precisions were reported (Table 10, 11, 12 & 13).

Table 10. Intra – Day Precision

Sr. No.	Label Claim (mg/tab)			Amount Found (mg/tab)			% of Label Claim		
	SER	DCL	PCM	SER	DCL	PCM	SER	DCL	PCM
1	10	17.308	100	9.93	17.5	100	99.3	101	100
2	10	17.308	100	10.00	17	99.72	100	98.22	99.72
3	10	17.308	100	10.4	17	100	104	98.22	100
4	10	17.308	100	9.93	17.5	98.33	99.3	101	98.33
5	10	17.308	100	10.00	17.5	102.77	100	101	102.77
6	10	17.308	100	9.93	17.5	101.94	99.3	101	101.94

Table 11. Statistical Validation for Intra – Day Precision

Drug	% Mean*	S.D*	% R.S.D.*	S.E.*
SER	100.31	1.83	1.82	0.7472
DCL	100.07	1.43	1.42	0.5839
PCM	100.46	1.61	1.60	0.6574

n=6

Table 12. Inter – Day Precision

Sr. No.	Label Claim (mg/tab)			Amount Found (mg/tab)			% of Label Claim		
	SER	DCL	PCM	SER	DCL	PCM	SER	DCL	PCM
1	10	17.308	100	9.93	17.5	100	99.3	101	100
2	10	17.308	100	10.4	17	99.72	104	98.22	99.72
3	10	17.308	100	10.0	17	98.33	100	98.22	98.33
4	10	17.308	100	10.0	17.5	101.94	100	101	101.94
5	10	17.308	100	10.0	17.5	102.77	100	101	102.77
6	10	17.308	100	9.93	17.5	98.33	99.3	101	98.33

Table 13.Statistical Validation for Inter – Day Precision

Drug	% Mean*	S.D.*	% R.S.D.*	S.E.*
SER	100.43	1.78	1.77	0.7268
DCL	100.07	1.43	1.42	0.5839
PCM	100.18	1.83	1.82	0.7472

n=6

The standard deviation (S.D.), relative standard deviation (%R.S.D.) and standard error (S.E.) calculated are low indicating high degree of precision of the method. The %R.S.D. is less than 2% as required by USP and ICH guidelines.

In current study first order derivative spectrophotometric method was performed.In first order derivative spectrophotometric absorbance method of Serratiopeptidase, Diclofenac potassium and Paracetamol were found on different wavelength which was used as working wavelength for particular drug. For Serratiopeptidase and Paracetamol working wavelength were selected 252 nm and 276 nm where absorbance of Diclofenac potassium was found zero. For Diclofenac potassium absorbance of drug was found at 330 nm where the absorbance of Serratiopeptidase and Paracetamol was found zero.

Validation of pure powder drug, pure powder mixture and tablet formulation performed with different parameter in which linearity for Serratiopeptidase 2 µg/ml to 15µg/ml, for Diclofenac potassium 2 µg/ml to 30 µg/ml and for Paracetamol 2 µg/ml to 80µg/ml. Precision, repeatability and recovery study were performed.

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The standard deviation (S.D.), relative standard deviation (% R.S.D) and standard error (S.E.) calculated are low indicating high degree of precision of the method. The %R.S.D. is less than 2% as required by USP and ICH guidelines.

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